

Applicants: Rolando Pajon Feyt, et al.  
Serial No.: 10/580,888  
Filing Date: May 25, 2006  
Docket No.: 976-33 PCT/US/RCE  
Response to non-final office action issued March 13, 2009  
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### **Amendments to the Specification**

*Please delete the paragraph on page 12, lines 6-13, of the originally filed specification, and replace it with the following new paragraph.*

Protein identification based on mass spectrum data was carried out using the ProFound program (Zhang W and Chait BT. 2000. ProFound: an expert system for protein identification using mass spectrometric peptide mapping information. *Anal Chem* 72:2482-2489. <http://prowl.rochester.edu/cgi-bin/ProFound>). The search was subscribed to the genes and derived protein sequences contained in the SwissProt database (<http://www.ebi.ac.uk/swissprot/>) and NCBI (<http://www.ncbi.nlm.nih.gov>), considering the oxidation of methionines, deamidation and carboxyamidomethylation of cysteines as possible modifications to be encountered.

*Please delete the paragraph on page 12, lines 14-18, of the originally filed specification, and replace it with the following new paragraph.*

Identification of proteins based on the mass spectra was carried out with the MASCOT program (Perkins DN, et al. 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* 20:3551-3567. <http://www.matrixscience.com/>). Search parameters included cysteine modifications as well as oxidations and deamidations.

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*Please delete the paragraph starting on page 12, line 27, and ending on page 13, line 2, of the originally filed specification, and replace it with the following new paragraph.*

For the identification of the NMB0928 protein, a sequence homology search was done in the NCBI data base employing the BLAST program (Altschul SF, et al. 1990. Basic local alignment search tool. *J Mol Biol* 215:403-410, <http://www.ncbi.nlm.nih.gov/BLAST>). The results of this procedure indicated homology with, in addition to the corresponding protein in other serogroups of *Neisseria*, with the one in several microorganisms, including lipoprotein - 34 codified by the *nlpB* gene from *Escherichia coli*, identified in 1991. It is demonstrated that this protein is fractionated in the outer membrane proteoliposomes (Bouvier J, Pugsley A.P and Stragier,P. 1991. A gene for new lipoprotein in the *dapA-purC* interval of the *E. coli* chromosome. *J Bacteriol* 173(17):5523-31)

*Please delete the paragraph starting on page 14, lines 4-5 of the originally filed specification, and replace it with the following new paragraph.*

For the prediction of signal peptide the SignalP World Wide Web server (<http://www.cbs.dtu.dk/services/SignalP-2.0>) was employed.

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*Please delete the paragraph starting on page 16, lines 19-34 of the originally filed specification, and replace it with the following new paragraph.*

To analyze the conservation of the sequence of the gene codifying for the NMB0928 protein in the pathogenic species of the *Neisseria* genus a similarity search with the genomes of *Neisseria meningitidis* (serogroups A, B and C) and *Neisseria gonorrhoeae*, annotated in the NCBI data base, was done (NC\_003116.1, NC\_003112.1, NC\_003221, NC\_002946 SANGER\_135720|Contig1) employing the BLAST program (Altschul SF, et al. 1990. Basic local alignment search tool. *J Mol Biol* 215:403-410. <http://www.ncbi.nlm.nih.gov/BLAST/>). Figure 8 shows the results of the sequence comparison for those sequences that produce a significant alignment in each of the analyzed genomes. Those sequences have 98% identity in serogroups A and C, 99% identity in serogroup B and 96% identity with *Neisseria gonorrhoeae*, with the sequence obtained for the gene that codifies for the NMB0928 protein (Seq. ID. No. 3). In addition, the sequence of the referred gene was determined for 3 Cuban isolates (Seq. ID. No. 5-7), which belong to serogroup B (B:4:P1.19,15) and a sequence alignment was done by using the ClustalX program (<http://www.ebi.ac.uk/clustalw/>). The results of the alignment show that there is a great conservation in the nucleotide sequence of the gene NMB0928 among the analyzed strains.